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Molecular docking reveals fibrinogen binding sites on SARS-CoV-2 spike protein: A potential mechanism for COVID-19-related coagulation

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Abstract

The Coronavirus Disease (COVID-19) pandemic, caused by Severe Acute Respiratory Syndrome coronavirus-2 (SARS-CoV-2), highlighted significant gaps in our understanding of the virus's molecular structure, its biological properties, and the interactions between viral proteins and host biological systems, which have underscored the critical need for further research in this area. Coagulation disorders, particularly those leading to thromboinflammation, have been linked to severe complications in COVID-19 cases. The occurrence of thromboinflammation has likewise been corroborated in cases of both Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS). In this study, we investigated the protein-protein interactions between the SARS-CoV-2 spike protein and fibrinogen, a glycoprotein that plays a crucial role in blood coagulation. Molecular docking analysis revealed key interactions between the β and γ subunits of fibrinogen and the spike protein. The findings suggest that these interactions may contribute to the understanding of the coagulation disorders observed in COVID-19 patients. This study provides insights into the molecular mechanisms underlying these disorders and identifies potential targets for the development of therapeutic interventions.

Keywords: Spike protein, fibrinogen, coagulation, molecular docking, molecular structure, severe acute respiratory syndrome coronavirus

Introduction

Coronaviruses are a large and diverse family of viruses that have demonstrated their impact by causing diseases with various forms throughout history [1]. In 2002 and 2012, relatively pathogenic coronaviruses of zoonotic origin, namely Middle East Respiratory Syndrome coronavirus (MERS-CoV) and Severe Acute Respiratory Syndrome coronavirus (SARS-CoV), caused deadly respiratory illnesses in human populations and led to the emergence of coronaviruses as a significant public health threat in the twenty-first century [2]. In late 2019, a new coronavirus known as SARS-CoV-2 was detected in Wuhan, China, leading to an unprecedented outbreak of viral pneumonia. This new coronavirus disease is known as Coronavirus Disease (COVID-19), which has a very high transmission capacity, spread rapidly worldwide, and caused a major health crisis [3]. Given the widespread geographic impact of the pandemic and the high number of individuals affected, COVID-19 has far surpassed the MERS and SARS pandemics in magnitude. The ongoing COVID-19 pandemic is a major threat to global human health, further emphasizing the need for precautions and research studies [4].

Data from numerous studies conducted during this pandemic indicate that hemostatic disorders such as hypercoagulability and hypofibrinolysis have been observed, primarily in severely ill patients receiving advanced care [5]. The prothrombotic effects of SARS-CoV-2 appear to be driven not by inherent biological factors but by inflammation and an endotheliopathic reaction [6]. The primary cause of morbidity and mortality in these patients is the activation of inflammatory responses and coagulation pathways, a phenomenon known as thromboinflammation [7]. Preliminary clinical observations from Wuhan, China, indicated that individuals with severe manifestations of the illness experienced acute pulmonary distress and hypoxemia [8]. Additional data from

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various countries have demonstrated that critically ill patients in intensive care often encounter thrombotic complications such as pulmonary embolism (PE) and deep vein thrombosis (DVT) [9,10].

The basic mechanisms underlying the hemostatic disorders associated with COVID-19 have not been characterized yet despite studies. Nevertheless, it is well-established that this phenomenon results from a profound inflammatory reaction to the virus, commonly referred to in the literature as 'thromboinflammation [10]. The occurrence of thromboinflammation has likewise been corroborated in cases of both SARS and MERS [11]. One notable result was the observation that abnormal clotting was not restricted to patients with acute COVID-19. It was also observed that stroke, pulmonary embolism or sudden death occurred in young COVID-19 patients with asymptomatic infections or mild respiratory symptoms [12]. In conclusion, with the presence of many factors that activate the coagulation system in patients, changes occurring in the entire pathologic structure cause multiple organ failure and impairment of the respiratory system, leading to high levels of mortality and morbidity [13]. For these reasons, in this study, our objective was to examine the interaction of fibrinogen glycoprotein, which is produced in the liver and plays a fundamental role in the blood coagulation process by being found in the blood plasma, with SARS-CoV-2 spike protein through molecular modeling [14].

Human fibrinogen, which is present in the circulation of our body and has a size of approximately 340 kDa, is an important glycoprotein synthesized by hepatocytes (Figure 1). It has a dimeric structure consisting of three different polypeptide chains, Aα, Bβ and γ [15]. When fibrinogen is exposed to thrombin, the α-subunit releases two fibrinopeptides called fibrinopeptide A (FPA). Similarly, the β-subunit releases two subunits called fibrinopeptide B (FPB) in the continuation of FPA release [14]. Fibrin monomers are formed when thrombin breaks down FPA and FPB. The monomers formed in this way can provide the formation of a fibrin network by bonding. Investigations into the interactions of SARS-CoV-2 with fibrinogen are limited. Recent studies have revealed that only spike protein and fibrinogen can interact and thus SARS-CoV-2 can affect coagulation by manipulating fibrin formation [12].

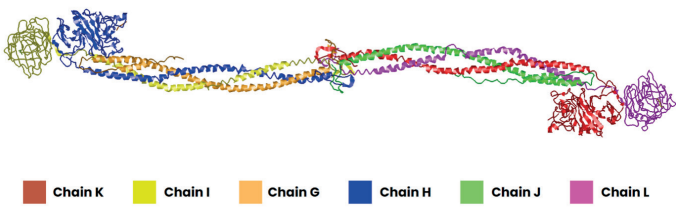

Figure 1. Schematic representation of the chain structures of monomerized human fibrinogen protein; (PDB: 3GHG) It consists of Aα (Chain G and J), Bβ (Chain K and H) and γ (I and L) polypeptide chains; the image of the protein was generated using VMD

SARS-CoV-2 has a highly functional and complex structure. The genome of coronaviruses (CoVs) comprises single-stranded, positive-sense RNA (+ssRNA), which is notably larger than that of other RNA viruses, ranging from 27 to 32 kilobases in length. The nucleocapsid protein (N) encases the genome within a core structure, while the genome is enveloped by a lipid membrane that incorporates three structural proteins: the membrane protein (M), the spike protein (S), and the envelope protein (E) [16]. SARS-CoV-2 possesses 4 structural proteins (S, E, M, and N) and sixteen non-structural proteins, ranging from nsp1 to nsp16. Spike proteins, which were studied in this study to examine protein-protein interactions, enter host cells through the spike glycoprotein (S protein) (Figure 2) [17]. The spike glycoprotein is essential for the viral entry of coronaviruses, positioning it as a significant target for antiviral strategies. This glycoprotein is composed of two functional subunits: S1 and S2. The S1 subunit includes the receptor-binding domain (RBD) and the N-terminal domain (NTD), and its primary role is to interact with the receptor on the host cell. The S2 subunit comprises several key elements: heptad repeat 1 (HR1), the fusion peptide (FP), the central helix (CH), the binding domain (CD), the transmembrane domain (TM), heptad repeat 2 (HR2), and the cytoplasmic tail (CT). The S2 subunit is responsible for mediating the fusion of the viral and host cell membranes [18]. In addition to the importance of knowing the spike structure, protein-protein interactions are crucial for predicting the functional roles of target proteins as well as assessing the potential efficacy of therapeutic molecules [19]. For this purpose, spike protein and fibrinogen were selected as the proteins whose interactions will be examined in our study. We aimed to reveal which regions and which amino acid residues of fibrinogen interact with the prediction-oriented biostatistical molecular docking method.

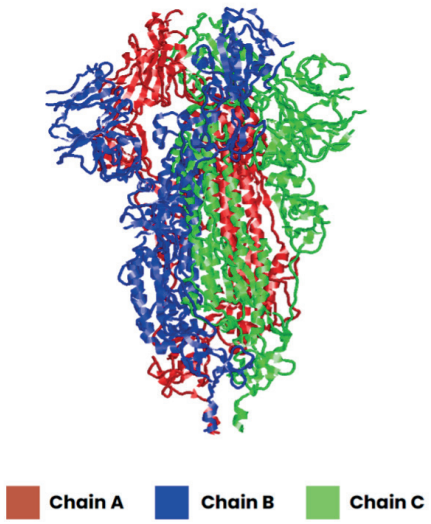

Figure 2. Schematic representation of the chain structures of the SARS-CoV-2 spike protein in its closed conformation; each chain contains both the S1 and S2 subunit; the image of the protein was generated using VMD

Material and Methods

Docking Preparation of the Proteins

The crystallographic structures of fibrinogen (PDB: 6GHG) and the SARS-CoV-2 spike protein (PDB: 6VXX) were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB). Prior to docking, geometry optimization of the proteins was performed using University of California, San

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Francisco (UCSF) Chimera on a Windows 10 operating system. For energy minimization, metals, water molecules, and unwanted chains were first removed from the structure using AutoDock Tools. Missing hydrogen atoms and charges were identified, and any missing residues or atoms that were not resolved during crystallization were repaired. After these steps, the proteins were ready for docking.

Molecular Docking

Protein-protein docking simulations were conducted using ClusPro 2.0, an automated server for rigid-body docking [20-23]. ClusPro generates 109 docking conformations by applying rotational and translational movements of one protein (referred to as the 'ligand') relative to another protein (designated as the 'receptor,' which remains fixed). These docking conformations are ranked based on their clustering characteristics. The scoring function integrates van der Waals interaction energy, electrostatic energy, dissociation energy contributions, and both attractive and repulsive forces in pairwise interaction potentials [23]. The 1,000 most energetically favorable docking conformations are organized into clusters, typically ranging from 10 to 30 clusters. To achieve this, the program employs a pairwise RMSD-based clustering technique to categorize the docking conformations and identify the largest representative clusters of the complex, as detailed in previous studies [21]. The binding scores obtained as a result of the docking process were analyzed and the most appropriate model was selected.

Analysis and Visualization of Results

The docking results were analyzed using the PDBsum database, and potential interactions were visualized through diagrams. Generally, PDBsum functions as a bioinformatics database that contains three-dimensional models of protein structures. This web-based tool also allows for the creation of diagrams that visually summarize a protein's sequence information, structural motifs, secondary structures, and ligand interactions. Additionally, it provides information about protein-protein interactions and their biological functions, which was utilized in our study [24-28]. Moreover, LigPlot+ software was used to generate 2D diagrams illustrating detailed interactions [29]. Visualization of the docking results was carried out using VMD [30].

Results

Molecular Docking Results

When the docking results were analyzed, approximately 20 poses were obtained in a single cluster. These poses were analyzed by representing them in two ways as “Center” and “Lowest Energy”. The energy value of the pose representing the average structure of the cluster was found to be -894 kcal/mol. This pose reflects the general trend of the cluster. The binding energy of the model with the lowest, i.e. the most stable and most probable binding position was obtained as-978.1 kcal/mol. This pose indicates that protein-protein interactions may be biologically relevant. In this study, the model with the best results was selected and visualizations were made based on this model. The poses gathered in a single cluster indicate the presence of a distinct binding pattern (Figure 3). When the results were analyzed, it was observed that the predominantly B chain of the Spike protein interacted with the K and I chains of fibrinogen. The K and I chains represent the chains of the β and γ subunits, respectively. Likewise, interactions were also established with the L chain, which is also located in the γ subunit, but considering the size, number and types of interactions, analyzes were made mainly on the interactions established with the K and I chains.

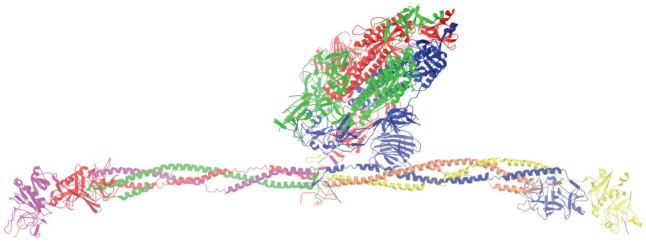

Figure 3. Visualization of the molecular docking result of human fibrinogen protein and SARS-CoV-2 spike protein; color representation is based on chain types; docking was performed through the web-based ClusPro 2.0 server and the image was acquired with VMD

Summary of Fibrinogen-Spike General Interaction

When the interactions between SARS-CoV-2 spike glycoprotein and fibrinogen were analyzed, we tried to focus on the interactions between the proteins as well as the interactions between each other. For this purpose, a general interaction diagram was drawn (Figures 4-6) and potentially important interactions were selected from this diagram and score sheets (Table 1).

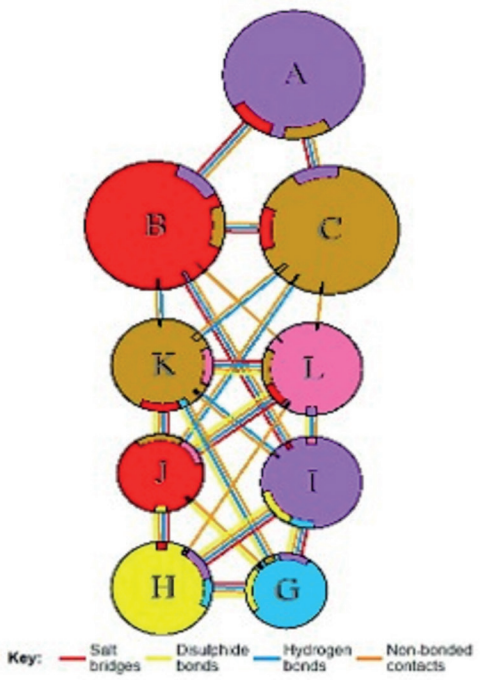

Figure 4. A schematic representation of interactions between protein chains is provided; ,nteracting chains are connected by colored lines, each denoting a distinct type of interaction according to the legend above; the size of each circle is proportional to the surface area of the respective protein chain; the extent of the interface region on each chain is illustrated by a colored wedge, where the color matches that of the interacting chain, and the size of the wedge indicates the surface area of the interface; interactions were analyzed using PDBsum, a web-based tool and database

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Analysis of Interactions with the β and γ Subunits of Fibrinogen

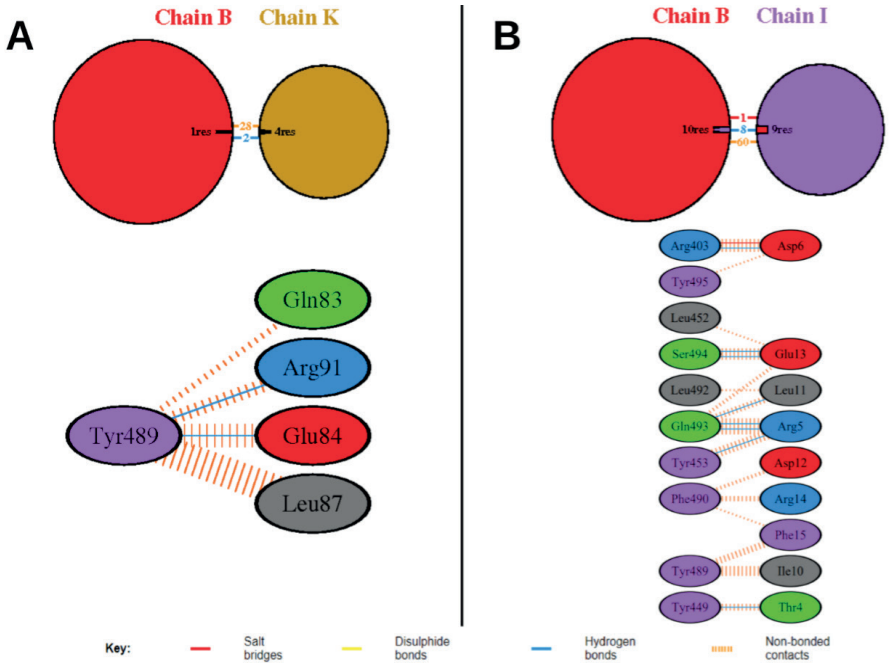

Figure 5. Schematic representation of the interactions of the B chain of the Spike protein with the K and I chains forming the β and γ subunits of fibrinogen; **A.** Illustration of the interactions between chain B and the K chain (β subunit), **B.** Illustration of the interactions between chain B and the I chain (γ subunit); types of bonds formed are represented with the following residue colors: positive residues (H, K, R) are shown in one color, negative residues (D, E) in another, neutral residues (S, T, N, Q) in a third, aliphatic residues (A, V, L, I, M) in a fourth, and aromatic residues (F, Y, W) in a distinct color; interactions were analyzed using PDBsum, a web-based tool and database

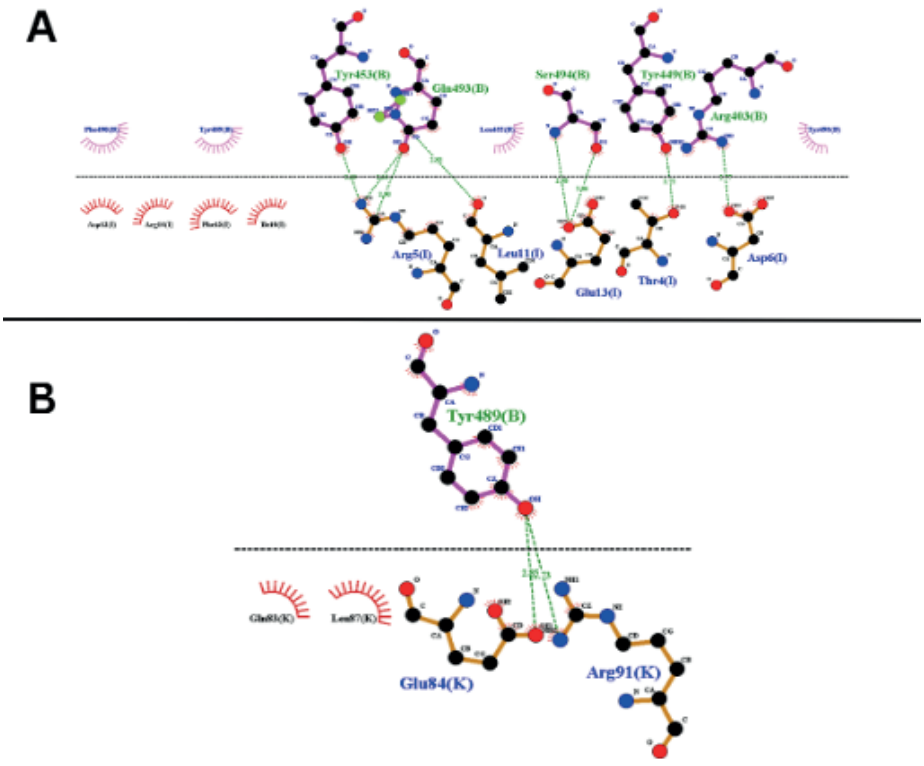

Figure 6. Chemical detailed and environmental representation of the interactions of the B chain of the Spike protein with the K and I chains forming the β and γ subunits of fibrinogen; **A.** Illustration of the interactions between the B chain and the I chain (γ subunit), **B.** Illustration of the interactions between the B chain and the K chain (β subunit); the distance of the bonds formed and the atoms with which they interact are given in more detail. In addition, the residues in the immediate vicinity are also shown; the analysis was conducted using LigPlot+ software

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Table 1. Tabular representation of protein-protein interactions comparing the number of interface residues, contact areas, salt bridges, disulfide bonds, hydrogen bonds and unbound contacts. Protein chains with larger surface area (such as Spike chains) are observed to interact more. Interactions were analyzed using PDBsum, a web-based tool and database

Chains	No. of interface residues	Interface area (Å²)	No. of salt bridges	No. of disulphide bonds	No. of hydrogen bonds	No. of non-bonded contacts
A-B	103:94	5014:5122	13	-	78	675
B-C	103:91	5055:5148	12	-	73	661
A-C	89:102	5146:5046	11	-	73	651
B-I	10:9	435:470	1	-	8	60
B-K	1:4	192:158	-	-	2	28
B-L	1:1	60:72	-	-	-	3
C-J	3:2	145:182	-	-	1	8
C-K	9:11	552:560	-	-	10	73
C-L	2:2	136:142	-	-	-	16
G-H	50:56	3182:3244	6	2	19	271
G-I	32:34	2004:2028	5	2	11	277
G-J	3:2	194:209	-	1	-	16
G-K	11:14	723:711	-	1	7	85
H-I	59:60	3334:3390	7	2	32	287
K-L	53:53	3314:3326	6	2	25	288
H-J	15:14	764:792	1	1	8	115
H-L	3:2	146:151	-	-	-	7
I-K	4:4	210:232	-	-	1	18
I-L	15:14	816:840	-	2	6	111
J-K	55:55	3405:3513	10	2	24	311
J-L	32:34	2110:2049	7	2	15	166

Discussion

Interactions between fibrinogen and the SARS-CoV-2 spike protein are scarcely documented in the literature. However, a recent study, which has not yet undergone peer review, revealed that the spike protein interacts with the β and γ subunits of fibrinogen [12]. This finding is consistent with our results and suggests that this interaction may play a critical role in the pathogenesis of COVID-19, particularly in the development of coagulation disorders such as thromboinflammation, which has been widely reported in severely ill patients [5,7].

Our molecular docking analysis further confirms that the SARS-CoV-2 spike protein establishes significant interactions with the β and γ subunits of fibrinogen. These findings imply that these interactions could contribute to the abnormal clot formation observed in COVID-19 patients. Specifically, we identified that peptides from the γ regions are spatially close to each other and engage with residues 386-394 located at the carboxy-terminal end of the epitope. This suggests the involvement of a three-dimensional conformational epitope within the γ-chain of fibrinogen (γC domain) in the interaction with the spike protein. Such interactions provide insight into a spike–fibrinogen-dependent mechanism of clot formation that may induce significant inflammatory and oxidative stress responses.

These results are in alignment with the findings of Ryu et al., who demonstrated that the SARS-CoV-2 spike protein can interact with fibrinogen, leading to the formation of abnormal inflammatory clots. Their study further suggests that these clots could be neutralized by fibrin-targeted immunotherapy, offering a potential therapeutic approach for managing COVID-19-related thromboinflammation. This aligns with our findings and further supports the notion that the spike protein not only facilitates viral entry but also plays a crucial role in triggering inflammatory responses and coagulation disorders through its interactions with host proteins.

Numerous structural, biophysical, and bioinformatics studies have identified the spike (S) protein as a critical target for protective antibodies, particularly the NTD and RBD of the S1 subunit, which are recognized as the principal immunogenic regions of the spike protein. However, there is limited data available regarding the antigenicity of the S2 subunit. The interaction of the RBD with the host cell has been extensively investigated as an attractive immunogenic target for vaccine development. In this study, we used prediction-oriented statistical analysis methods to suggest possible interaction sites between the spike protein and fibrinogen. For more detailed and accurate analyses, molecular dynamics simulations and laboratory studies are planned to confirm these binding sites in future research.

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In summary, the interaction between the SARS-CoV-2 spike protein and fibrinogen represents a significant aspect of COVID-19 pathophysiology. Our findings, along with those of other studies, highlight the potential for targeting these interactions in the development of therapeutic strategies. This understanding not only enhances our knowledge of the molecular mechanisms underlying COVID-19-related coagulation disorders but also opens new avenues for treatment approaches aimed at mitigating the severe complications associated with this disease.

Conclusion

Protein-protein interactions play a crucial role in drug development, disease understanding, and the discovery of new biological properties. Specifically, studying the interactions of the SARS-CoV-2 spike glycoprotein, which has recently caused a global pandemic, can lead to the identification of novel properties and the development of new treatment strategies.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical Approval
This research is a software-based modeling study using open access Protein Data Bank (PDB) data. Ethical approval is therefore not required.

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B.G.İ. and E.T. planned and carried out the simulations. O.Ö., B.G.İ. and E.K. wrote the manuscript with input from all authors. O.Ö. and E.K. contributed equally, were involved in planning and supervised the work.

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